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(12) **Patent:** (11) **CA 951662**

(54) PROCESS FOR PREPARING SUBSTITUTED PHENYLALKANOIC ACIDS

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ABSTRACT:

CLAIMS: Show all claims

*** Note: Data on abstracts and claims is shown in the official language in which it was submitted.

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Important Notices

PROCESS FOR PREPARING SUBSTITUTED PHENYLALKANOIC ACIDS

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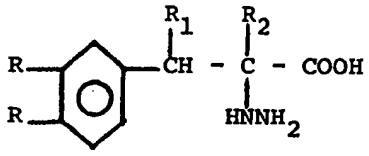
This invention describes a new method of preparing certain α-hydrazino-β-phenylalkanoic acids and their derivatives. More particularly, it describes a method of preparing L-α-hydrazino-β-hydroxyphenyl alkanoic acids.

It is known in the art that various α-hydrazino-β-phenylalkanoic acids are useful as decarboxylase inhibitors. It is further known that the D-isomer of these acids is generally inactive and may even be antagonistic to the action of the L-form, thereby reducing its potency.

This invention describes a novel process for the preparation of the L-α-hydrazino-β-phenylalkanoic acids.

The present invention provides a new method of preparing the L-stereoisomeric compounds of Formula I:



I

where

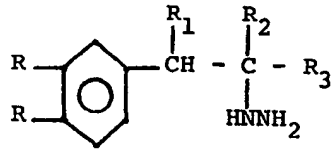
R is hydrogen or hydroxy;

R_1 is hydrogen or lower alkyl; and

R_2 is hydrogen or lower alkyl.

It is to be understood that the L-configuration is in reference to the absolute configuration on the α-carbon in relation to the hydrazine.

We have found that the compounds of Formula I can be conveniently prepared by microbiological hydrolysis of the L-stereoisomeric compound of Formula II:



II

where

R_3 is loweralkoxy carbonyl,

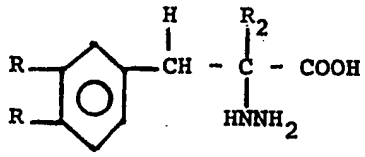
amido or

cyano; and

R, R_1 and R_2 are as described above.

We have found that the compounds of Formula II can be contacted with specific enzymes which cause hydrolysis to the desired L-stereoisomeric acid of Formula I and thereby eliminate costly and complicated separation procedures.

A more preferred embodiment of this invention describes the preparation of the L-stereoisomeric compound of Formula III:



III

where R and R_2 are as described above.

A most preferred embodiment of this invention describes the preparation of L-α-(3,4-dihydroxybenzyl)-α-hydrazino propionic acid and L-β-(3,4-dihydroxyphenyl)-α-hydrazino propionic acid.

In the above descriptive portions of formulae I - III, the following definitions apply:

The "lower alkoxy" radical signifies an alkoxy group containing from 1 to about 6 carbon atoms which can be straight chained or branched.

"Aralkoxy" refers to an arylalkoxy group, the aryl portion of which may be one or more phenyl or naphthyl radicals attached to an α-alkoxy radical which contains from 1 to about 4 carbon atoms. The preferable aralkoxy groups are benzyl, diphenylmethyl, trityl, naphthylmethyl and substituted benzyl and the like groups. Such substituents may include lower alkyl such as o-methylbenzyl, lower alkoxy such as 3,4-veratryl and 4,4',4"-trimethoxytrityl and the like.

The present invention may be practiced by intimately contacting an enzyme system capable of hydrolyzing an ester, amide or nitrile compound of Formula II to form the desired acid.

The esters of Formula II may be hydrolyzed to the free acid by a wide variety of enzymatic systems which are capable of selectively hydrolyzing the ester group. Thus, for example, methyl L-α-(3,4-dihydroxybenzyl)-α-hydrazinopropionate may be saponified by pig liver esterase to yield L-α-(3,4-dihydroxybenzyl)-α-hydrazinopropionic acid. Other microorganisms which may selectively hydrolyze the ester compounds of Formula II include *Aspergillus oryzae*, *Zygosaccharomyces acidifiens*, *Streptomyces spheroides* and *Alcaligenes sp.* These microbiological systems may be used *in situ* or the cell-free broth may be used as the enzyme source. The cells can be used as suspensions in pH 5.0 to pH 9.0 phosphate buffer at levels equivalent to 0.1 to 10% dry weight basis. A cell-free system may be prepared by sonication of the cells followed by centrifugation.

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The ester is dissolved in aqueous methanol solution and used at levels such that the final substrate concentration in the enzyme solution will be between 0.1 to 10%. The final methanol concentration may range from 10 to 30%.

The nitriles of Formula II may also be hydrolyzed to the free acid by enzymatic hydrolysis in the presence of a nitrilase. The hydrolysis may be carried out by using nitrilase which is found in the leaves of barley, oats, cabbage, cauliflower, brussel sprouts, etc. This enzyme has the power to hydrolyze nitriles directly to the carboxylic acids. The nitrilase is extracted with 0.1 M phosphate buffer at pH 7.5 from the leaves of oats or barley. Ten to 50 g. are obtained per 100 ml. of buffer. The nitrile and enzymes are dissolved in methanol to give 10 - 20% methanol in the final 0.1 - 10% enzyme-nitrile substrate mixture. Hydrolysis is carried out by stirring at room temperature, keeping the pH at 7 - 8 by the addition of alkali.

The amides of Formula II may also be hydrolyzed to the corresponding acid of Formula I through the use of amide-hydrolyzing enzymes. Among those hydrolases that may be used are the deacylases, and the amidases such as urease. The hydrolyzing system may be used as the free enzyme cells or in an enzyme broth suspension of from 0.1 to 10% concentration. The hydrolysis is usually carried out in a pH medium near neutrality but may be carried out at pH 5 - 9 usually with a phosphate buffer. The amide is dissolved in aqueous methanol solution and used at levels such that the final substrate concentration in the enzyme solution will be between 0.1 to 10%. The final methanol concentration may range from 10 - 30%.

If desired two enzymatic reactions may proceed at the same time. Thus, for example, L-α-N^1-acetylhydrazino-α-(3,4-dihydroxybenzyl)propionitrile may be subjected to a nitrilase and an amidase and result in the desired L-α-hydrazino-α-(3,4-dihydroxybenzyl)propionic acid.

The following examples are illustrations of this invention. They are intended to representatively show the method of this invention and are not to be construed as limitations thereof.

EXAMPLE 1

To L-α-(3,4-dihydroxybenzyl)-α-ureidopropionic acid [J. Med. Chem., 7, 379 (1964)] (25.4 g., 0.1 mole) in 150 ml. of methanol is added 16.74 g. (0.31 mole) of sodium methylate. With stirring 23.32 g. (0.2 mole) of benzyl chloride is added dropwise and the mixture refluxed for 4 hours. Water (200 ml.) is added and the mixture is distilled until the boiling point reaches 95-98°. By addition of hydrochloric acid the pH of the mixture is adjusted to 3.5, the volume adjusted to 200 ml. by addition of water and the mixture refluxed for 2 hours additional. After water is removed in vacuo, the mixture is extracted with methanol and the methanol extract chromatographed over silica gel. The eluate is concentrated to obtain L-α-(3,4-dibenzyloxybenzyl)-α-ureidopropionic acid.

To an ice-cold mixture of the hydantoic acid of the previous step (21.78 g., 0.05 mole) in 100 ml. of 2.5 N sodium hydroxide is added to a solution of sodium hypochlorite (89.5 ml., 0.70 N, 0.0625 mole). After the addition is completed the mixture is stirred for 5 minutes at 0-5°. The mixture is then heated to 80° and maintained at 80° for 1.5

hours. At the end of this period 300 ml. of toluene and 4 ml. of 85% hydrazine hydrate are added and the mixture vigorously agitated while 54 ml. of concentrated hydrochloric acid is added. The mixture is stirred at 80° for 30 minutes, the phases separated and the aqueous phase washed with 150 ml. of toluene. The aqueous phase is evaporated to dryness, the residue extracted with methanol and the pH of the extract adjusted to 6.4 with diethylamine. The product is separated by filtration and recrystallized from methanol-water to obtain L-α-(3,4-dibenzyloxybenzyl)-α-hydrazinopropionic acid. To a solution of L-α-(3,4-dibenzyloxybenzyl)-α-hydrazinopropionic acid (8.13 g., 0.02 mole) dissolved in 100 ml. of methanol is added hydrogen chloride gas until the saturation point is reached. The mixture is stirred at room temperature (25°) for 24 hours and then evaporated to dryness *in vacuo*. The residue is crystallized from methanol-water to yield methyl L-α-(3,4-dibenzyloxybenzyl)-α-hydrazino propionate.

A solution of methyl L-α-(3,4-dibenzyloxybenzyl)-α-hydrazinopropionate (8.4 g., 0.02 mole) in 200 ml. of acetic acid is hydrogenated over 0.5 g. of 5% palladium on charcoal at room temperature and 3 atm. pressure. The catalyst is removed by filtration, washed and the filtrate concentrated to yield methyl L-α-(3,4-dihydroxybenzyl)-α-hydrazinopropionate.

A solution of methyl L-α-(3,4-dihydroxybenzyl)-α-hydrazinopropionate (9.6 g., 0.04 mole) in 1 l. of 0.01 M potassium chloride was brought to pH 8.0 by addition of potassium hydroxide from a microburet. Approximately 100 units of pig liver esterase was added and allowed to act at 37°. Potassium hydroxide was added as required to maintain

the pH at 8.0. After 4 hours the pH is adjusted to 6.4 with hydrochloric acid and the mixture concentrated in vacuo to dryness. The residue is extracted with methanol and the methanol extract is concentrated in vacuo to dryness. On recrystallization from water L-α-hydrazinopropionic acid, m.p. 208° dec. is obtained.

EXAMPLE 2

A solution of methyl L-α-(3,4-dihydroxybenzyl)-α-hydrazinopropionate (9.6 g., 0.04 mole) in 1 l. of 0.01 M potassium chloride is brought to pH 8.0 by addition of potassium hydroxide from a microburet. Approximately 100 units of pig liver esterase [J. Am. Chem. Soc., 84, 695 (1962)] is added and allowed to act at 37°C. Potassium hydroxide is added as required to maintain the pH at 8.0. After 4 hours the pH is adjusted to 6.4 with hydrochloric acid and the mixture concentrated in vacuo to dryness. The residue is extracted with methanol and the methanol extract is concentrated in vacuo to dryness. On recrystallization from water L-α-hydrazinopropionic acid, m.p. 208°C. dec. is obtained.

When methyl L-α-(3,4-dihydroxybenzyl)-α-hydrazinopropionate is replaced in the above example by the compounds of Table I, then the compounds of Table II are prepared.

TABLE I

methyl L-α-(3-hydroxybenzyl)-α-hydrazinopropionate,

methyl L-β-(3,4-dihydroxyphenyl)-α-hydrazinopropionate,

methyl L-β-(3,4-dihydroxyphenyl)-α-hydrazinobutanoate,

methyl L-(4-hydroxybenzyl)-α-hydrazinopropionate,

methyl L-β-(3,4-dihydroxyphenyl)-α-methyl-α-hydrazinobutanoate

TABLE II

L-α-(3-hydroxybenzyl)-α-hydrazinopropionic acid,

L-β-(3,4-dihydroxybenzyl)-α-hydrazinopropionic acid,

L-β-(3,4-dihydroxyphenyl)-α-hydrazinobutanoic acid,

L-α-(4-hydroxybenzyl)-α-hydrazinopropionic acid,

L-β-(3,4-dihydroxyphenyl)-α-methyl-α-hydrazinobutanoic acid.

EXAMPLE 3

Ethyl L-α-(3,4-dihydroxybenzyl)-α-hydrazinopropionate is dissolved in aqueous methanol solution and used at levels such that the final substrate concentration in the enzyme solution will be between 0.1 to 10%.

The system, capable of hydrolyzing the ethyl L-α-(3,4-dihydroxybenzyl)-α-hydrazinopropionate is selected from the microorganisms - *Aspergillus oryzae, Zygosaccharomyces acidifiens, Streptomyces spheroides* and *Alcaligenes sp.* The cells or the cell-free broth may be used as the enzyme source. The cells are suspended in pH 5.0 to pH 9.0 phosphate buffer at levels equivalent to 0.1 to 10% dry weight basis. The cell-free system may be prepared by sonication of the cells followed by centrifugation.

The final methanol concentration is from 10 - 30%.

The mixture is stirred at 37°C. for 1 - 6 hours. Dilute alkali is added periodically in order to maintain the pH near neutrality. The product is isolated by filtration from the insoluble material followed by acidification of the filtrate to pH 1 - 2 with 1 N hydrochloric acid. Concentration *in vacuo* results in crude L-α-(3,4-dihydroxybenzyl)-α-hydrazinopropionic acid hydrochloride. The crude acid salt is adsorbed on Amberlite®-IR-120 on the acid ($H_3^{\oplus}O$) cycle. The product is eluted with 1 N ammonium hydroxide, the

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eluate concentrated to dryness *in vacuo* and recrystallized from water containing 0.5% sodium bisulfite to yield the product of this invention, m.p. 208° dec.

EXAMPLE 4

By the procedure of Canadian Patent No. 791,154, July 30, 1968, L-α-3,4-dihydroxy-α-methylphenylalanine is converted successively to L-4(3',4'-diacetoxybenzyl)-2,4-dimethylazlactone, L-α-acetamido-α-3-acetoxy-4-hydroxybenzyl-propionamide and L-α-acetamido-α-(3,4-diacetoxybenzyl)propio-
10 nitrile.

To a slurry of 100 ml. of water, 200 ml. of ether, 36 ml. of concentrated hydrochloric acid and (0.25 mole) of L-α-acetamido-α-(3,4-diacetoxybenzyl)propionitrile at 0-10° is added dropwise with vigorous stirring 18 g. (0.26 mole) of sodium nitrite in 36 ml. of water. The temperature is maintained at 0-10° during addition and during one hour of stirring. The ether layer is separated, the water layer extracted with two 100 ml. portions of ether, the combined ethereal extract is washed with saturated salt solution and
20 the ethereal extract dried ($MgSO_4$). The mixture is concentrated *in vacuo* to yield L-α-N-acetyl-N-nitroso-α-(3,4-diacetoxybenzyl)propionate.

A mixture of 65.5 g. (1.0 mole) of zinc dust and 100 ml. of water is cooled to 10°. While stirring (0.24 mole) of nitroso compound in 150 ml. of glacial acetic acid is added while maintaining the temperature at 10-15°. After addition is finished the mixture is allowed to warm to room temperature over an hour and then warmed to 80° on the steam bath. The mixture is filtered to remove unreacted zinc, and the precipi-
30 tate washed with three 25 ml. portions of warm 2 N hydro-

chloric acid. The combined filtrate is cooled to room temperature and with cooling basified to pH 6.5. The mixture is filtered and the precipitate dried. The residue is extracted with three 200 ml. portions of chloroform. The dried ($MgSO_4$) extract is concentrated *in vacuo* to a residue which is recrystallized from methanol to yield L-α-N^1-acetylhydrazino-α-(3,4-diacetoxybenzyl)-propionitrile.

The compound (33.34 g., 0.1 mole) of the previous step is refluxed with 1650 ml. of methanol for 5 hours in the presence of sodium bicarbonate (16.6 g., 0.2 mole). The ice-cold mixture is brought to pH 7.6 with cold 1 N hydrochloric acid and concentrated to dryness *in vacuo*. The residue is extracted with ethanol, the ethanol extract concentrated to dryness and the residue triturated with isopropyl alcohol to yield L-α-N^1-acetylhydrazino-α-(3,4-dihydroxybenzyl)propionitrile.

The acetylhydrazinonitrile (12.46 g., 0.05 mole) of the previous step is subjected to an amidase to yield L-α-(3,4-dihydroxybenzyl)-α-hydrazinopropionitrile.

EXAMPLE 5

The nitrilase enzyme used is extracted with 0.1 M phosphate buffer, pH 7.5 from the leaves of oats (Avena), barley (Honderum), the Brossica-family and certain Musaceve. Ten to 50 g. of the material are extracted per 100 ml. of buffer.

L-α-(3,4-dihydroxybenzyl)-α-hydrazinoacetonitrile is dissolved in a quantity of methanol such that the final concentration of methanol is between 10 - 30% in the enzyme-substrate mixture. The final substrate concentration is kept between 0.1 to 10%. The hydrolysis is carried out at 25 - 37°C.

for 1 - 6 hours. The pH is maintained at 7.0 - 8.0 by the addition of dilute alkali. At the end of this time, the mixture is acidified to pH 1 - 2 with dilute HCl and extracted with chloroform. The aqueous extracts are washed with chloroform and concentrated in vacuo to dryness leaving a residue of crude L-β-(3,4-dihydroxyphenyl)-α-hydrazinopropionic acid hydrochloride and purified as in Example 3, m.p. 208° dec.

When L-α-(3,4-dihydroxybenzyl)-α-hydrazinoacetonitrile of the above example is replaced by the compounds of Table III then the products obtained are shown in Table I.

TABLE III

L-α-(3-hydroxybenzyl)-α-hydrazinopropionitrile,

L-β-(3,4-dihydroxyphenyl)-α-hydrazinopropionitrile,

L-β-(3,4-dihydroxyphenyl)-α-hydrazinobutanonitrile,

L-α-(4-hydroxybenzyl)-α-hydrazinopropionitrile,

L-β-(3,4-dihydroxyphenyl)-α-methyl-α-hydrazinobutanonitrile.

EXAMPLE 6

20.72 G. (0.1 mole) of L-α-(3,4-dihydroxybenzyl)-α-hydrazinopropionitrile is stirred at 0-5°C. with 200 ml. of 45% hydrochloric acid for 2 hours. With stirring, the mixture is allowed to warm to room temperature, concentrated to dryness in vacuo to obtain L-α-(3,4-dihydroxybenzyl)-α-hydrazinopropionamide.

EXAMPLE 7

When the nitrile compounds of Example 3 are replaced with the corresponding amide compounds of Table IV and the nitrilase enzyme is replaced with an amide-hydrolyzing enzyme, then the corresponding products of Table I are prepared.

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TABLE IV

L-α-(3,4-dihydroxybenzyl)-α-hydrazinopropionamide,

L-α-(3-hydroxybenzyl)-α-hydrazinopropionamide,

L-β-(3,4-dihydroxyphenyl)-α-hydrazinopropionamide,

L-β-(3,4-dihydroxyphenyl)-α-hydrazinobutanamide,

L-α-(4-hydroxybenzyl)-α-hydrazinopropionamide,

L-β-(3,4-dihydroxyphenyl)-α-methyl-α-hydrazinobutanamide.

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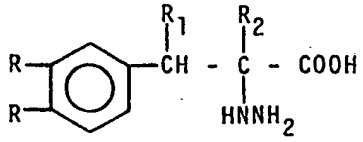
The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A process for the preparation of the L-stereoisomeric compound of the formula:



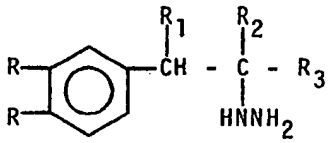
where

R is hydrogen or hydroxy;

R_1 is hydrogen or lower alkyl; and

R_2 is hydrogen or lower alkyl

which comprises hydrolyzing in a suitable medium at a pH of 5 to 9 the L-stereoisomeric compound of the formula:



where

R_3 is loweralkoxycarbonyl,

amido or

cyano; and

R, R_1 and R_2 are as described above,

by intimately contacting with a hydrolytic enzyme system selected from an esterase which is selected from pig liver esterase or a microorganism selected from *Aspergillus oryzae*, *Zygosaccharomyces acidifiens*, *Streptomyces spheroides* and *Alcaligenes* sp., an amide-hydrolyzing enzyme selected from deacylase and urease, and a nitrilase.

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2. A process according to Claim 1 where

R is hydroxy,

R_1 is hydrogen and

R_2 is hydrogen

thus forming L-β-(3,4-dihydroxyphenyl)-α-hydrazinopropionic acid.

3. A process according to Claim 1 where

R is hydroxy,

R_1 is hydrogen and

R_2 is methyl

thus forming L-β-(3,4-dihydroxyphenyl)-α-methyl-α-hydrazinopropionic acid.

4. A process according to Claim 2 or 3 where R_3 is loweralkoxycarbonyl and the hydrolytic enzyme system is an esterase selected from pig liver esterase or a microorganism selected from *Aspergillus oryzae*, *Zygosaccharomyces acidifiens*, *Streptomyces spheroides* and *Alcaligenes* sp.

5. A process according to Claim 2 or 3 where R_3 is amido and the hydrolytic enzyme system is an amide-hydrolyzing enzyme selected from deacylase and urease.

6. A process according to Claim 2 or 3 where R_3 is cyano and the hydrolytic enzyme system is a nitrilase.

ABSTRACT

A new method of preparing L-α-hydrazino-β-phenyl-alkanoic acids by enzymatic hydrolysis of the corresponding esters, amides and nitriles is disclosed. The esters are hydrolyzed by using an esterase such as pig liver esterase or a microorganism selected from *Aspergillus oryzae*, *Zygosaccharomyces acidifiens*, *Streptomyces spheroides* and *Alcaligenes* sp. The amides are hydrolyzed by using an amide-hydrolyzing enzyme selected from deacylase and urease while the nitrile is hydrolyzed by using nitrilase.

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